combination of SEs (nine SEs coded for by SaPIm1/n1 [18], *egc* [19], and *sep*). Among those, TSST-1 may suppress the mobility of polymorphonuclear neutrophils to infection sites [18], allowing MRSA to invade tissues. Moreover, since Spa protein binds to the tumour necrosis factor-α receptor of tissue cells [20], Spa protein, with a shorter stem, of strain NN33 (*spa*387) may allow MRSA to get closer to and damage tissue cells effectively.

In addition to the prematurity (i.e. ELBW) of the patient as a risk factor, a high-level combination of SEs (including TSST-1), together with adhesins and Spa (*spa*387 variant), could have contributed to the pathogenesis of fatal necrotizing fasciitis with sepsis and DIC.

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Transparency Declaration

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References

1. Miller LG, Perdreau-Remington F, Rieg G *et al.* Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *N Engl J Med* 2005; 352: 1445–1453.
2. McHenry CR, Piotrowski JJ, Petrinic D, Malangoni MA. Determinants of mortality for necrotizing soft-tissue infections. *Ann Surg* 1995; 221: 558–563.
3. Brook I, Frazier EH. Clinical and microbiological features of necrotizing fasciitis. *J Clin Microbiol* 1995; 33: 2382–2387.
4. Brumfitt W, Hamilton-Miller J. Methicillin-resistant *Staphylococcus aureus*. *N Engl J Med* 1989; 320: 1188–1196.
5. Zetola N, Francis JS, Nuermberger EL, Bishai WR. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect Dis* 2005; 5: 275–286.
6. Vandenesch F, Naimi T, Enright MC *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton–Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 2003; 9: 978–984.
7. Takano T, Higuchi W, Otsuka T *et al.* Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* strains belonging to multilocus sequence type 59 in Taiwan. *Antimicrob Agents Chemother* 2008; 52: 837–845.
8. Aires de Sousa M, Conceição T, Simas C, de Lencastre H. Comparison of genetic backgrounds of methicillin-resistant and -susceptible *Staphylococcus aureus* isolates from Portuguese hospitals and the community. *J Clin Microbiol* 2005; 43: 5150–5157.
9. Kuroda M, Ohta T, Uchiyama I *et al.* Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. *Lancet* 2001; 357: 1225–1240.
10. Takizawa Y, Taneike I, Nakagawa S *et al.* A Panton–Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. *J Clin Microbiol* 2005; 43: 3356–3363.
11. Otsuka T, Saito K, Dohmae S *et al.* Key adhesin gene in community-acquired methicillin-resistant *Staphylococcus aureus*. *Biochem Biophys Res Commun* 2006; 346: 1234–1244.
12. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2006; 193: 1495–1503.
13. Young LM, Price CS. Community-acquired methicillin-resistant *Staphylococcus aureus* emerging as important cause of necrotizing fasciitis. *Surg Infect (Larchmt)* 2008; 9: 469–474.
14. Hsieh WS, Yang PH, Chao HC, Lai JY. Neonatal necrotizing fasciitis: a report of three cases and review of the literature. *Pediatrics* 1999; 103: e53.
15. Dehority W, Wang E, Vernon PS, Lee C, Perdreau-Remington F, Bradley J. Community-associated methicillin-resistant *Staphylococcus aureus* necrotizing fasciitis in a neonate. *Pediatr Infect Dis J* 2006; 25: 1080–1081.
16. Hayani KC, Mathew R, Oyedele T, Hulten KG. Neonatal necrotizing fasciitis due to community-acquired methicillin resistant *Staphylococcus aureus*. *Pediatr Infect Dis J* 2008; 27: 480–481.
17. Schlievert PM, Bohach GA. Staphylococcal and Streptcoccal superantigens: an update. In: Kotb M, Fraser JD, eds, *Superantigens: molecular basis for their role in human diseases*. Washington, DC: ASM Press, 2007; 21–36.
18. Novick RP. Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of *Staphylococcus aureus*. *Plasmid* 2003; 49: 93–105.
19. Jarraud S, Peyrat MA, Lim A *et al. egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. *J Immunol* 2001; 166: 669–677.
20. Gómez MI, O'Seaghdha M, Magargee M, Foster TJ, Prince AS. *Staphylococcus aureus* protein A activates TNFR1 signaling through conserved IgG binding domains. *J Biol Chem* 2006; 281: 20190–20196.

A family outbreak due to an *emm*-type 11 multiresistant strain of *Streptococcus pyogenes*

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Abstract

Four cases of *Streptococcus pyogenes* infection due to an *emm*-type 11 strain, including one with a fatal outcome, occurred within a seven-member family. All strains shared biotype 5, pyrogenic exotoxin genes *spe*B and *spe*C, and resistance to kanamycin, tetracycline, macrolides and lincosamides. The identity of *Sma*I pulsed-field gel electrophoresis patterns confirmed their clonal origin. This highlights the ability of *S. pyogenes* to spread rapidly among family members. This first report of a family outbreak due to *emm*11 *S. pyogenes* reinforces the importance of surveillance of close family contacts of individuals with invasive streptococcal disease, and provides further support for antibiotic prophylaxis among the elderly.

Keywords: Bacteraemia, *emm*-type 11, outbreak, prophylaxis, streptococcal toxic shock syndrome, *Streptococcus pyogenes*

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Although community-acquired invasive infections due to *Streptococcus pyogenes* are increasing worldwide, the recently published outbreaks related to *emm*-type 11 were deemed to be hospital acquired [1,2]. We identified a multiresistant *emm*11 clone responsible for a family outbreak. The index case, an 87-year-old woman, was hospitalized in the intensive care unit for erysipelas of the leg associated with streptococcal toxic shock syndrome. A strain of *S. pyogenes* was isolated from blood and cutaneous swab. The patient received intravenous amoxicillin plus clavulanic acid 1 g/6 h for 7 days, combined for the first 2 days with ciprofloxacin 200 mg/12 h, and for the following 5 days with gentamicin 20 mg/8 h. She was discharged with a prescription of oral amoxicillin plus clavulanic acid 1 g/8 h for 15 days and recovered.

The 47-year-old daughter was hospitalized 7 days after her mother because of fever and acute abdominal pain. *S. pyogenes* was isolated from blood. A computed tomography scan showed a retroperitoneal peri-aortic abscess. She was treated with intravenous amoxicillin 3 g/6 h plus gentamicin 180 mg/day for 8 days. She then received an oral course of amoxicillin 2 g/8 h for 15 days and recovered uneventfully.

The 82-year-old husband of the index case had a medical history of coronary by-pass surgery and aneurysm of abdominal aorta. He was hospitalized 10 days after his wife with streptococcal toxic shock syndrome, and died several hours after admission. Blood cultures yielded the same *S. pyogenes* strain.

The 16-year-old grandson presented on the following day with pharyngitis due to the same strain. Throat swab specimens were not obtained from the remaining family members, two adults, 33 and 48 years old, and a 10-year-old girl, who were asymptomatic. They received an antibioprophylactic treatment.

Identification and typing techniques, performed as previously described, revealed that all the isolates were identical [3,4]. Biotype 5 was determined by the presence of β-glucuronidase and fermentation of methyl-β-D-glucopyranoside on rapid ID32 STREP strips (bioMérieux, Marcy l'Etoile, France). The strain was non T-typeable by slide agglutination with type-specific antisera (Sevapharma, Praha, Czech Republic). Antimicrobial susceptibility was tested by the disk diffusion method according to the Comité de l'Antibiogramme de la Société Française de Microbiologie guidelines (http://www.sfm.asso.fr). MICs were determined by E-test (AES, Chemuney, Bruz, France). The *mefA*, *ermB*, *ermTR*, *tet*O, *tet*M, *tet*K and *tet*L genes involved in macrolide or tetracycline resistance were searched by multiplex PCR [5]. The strain was resistant to tetracycline (MIC = 24 mg/L, in relation to the presence of the *tet*M gene). It had high levels of resistance to kanamycin (MIC $\geq$ 500 mg/L), erythromycin and clindamycin (MICs $\geq$ 256 mg/L in relation to the presence of the *erm*B gene). Genes encoding the toxins or superantigens SpeA, SpeB, SpeC and Ssa were determined by a multiplex PCR method [4]. The strain was positive for genes *spe*B and *spe*C. The identical *emm* sequence-type 11 and pulsed-field gel electrophoresis pattern of all the isolates, obtained as previously described, confirmed their clonal origin (Fig. 1) [3].

In Europe and in the USA *emm*11 strains are associated with invasive infections in < 5% of cases [6–9]. In France, for example, in 2007 the three most prevalent types (*emm*1, *emm*89, *emm*28) represented 60% of invasive strains. The association of *emm*-type 11 isolates and *erm*B gene has been

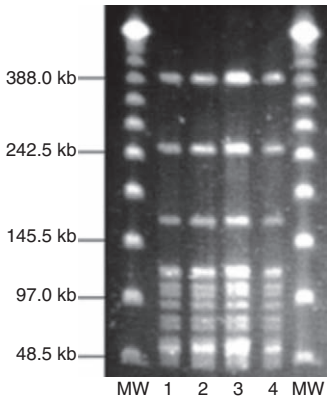

FIG. 1. Pulsed-field gel electrophoresis of *Sma*I-digested chromosomal DNA of the four isolates. MW, size marker (Lambda Ladder PFG Marker; New England Biolabs, Inc., Beverly, MA, USA). 1, 2, 3, and 4, *emm*- type 11 isolates from cases 1, 2, 3, and 4. kb, kilobases.

reported in France and in other European countries [7,10,11]. In Spain, an *emm*-type 11 clone harboured both *erm*B and *tet*M genes [12]. In *S. pyogenes*, tetracycline resistance is usually associated with the *tet*M gene and has been considered to be an important cofactor in the selection of erythromycin resistance [13]. In the period 2003–2004, higher rates of macrolide resistance were observed in France and Italy compared with nine other European countries participating in the Strep-EURO surveillance programme for invasive *S. pyogenes* infections, whereas tetracycline resistance was found in almost all participating countries [14]. High levels of resistance to kanamycin in *S. pyogenes* have been previously reported, associated with *emm*-type 28 multiresistant strains (characterized by high levels of resistance to bacitracin, erythromycin, clindamycin, kanamycin and streptomycin), but not with *emm*-type 11 strains [3].

The spread of *S. pyogenes* strains among the close contacts of infected persons is well recognized, especially in families including children [15,16]. In the reported cluster, the index case had erysipelas with positive blood cultures. The isolation of *S. pyogenes* from the cutaneous swab supports the theory of rapid transmission to family members sharing accommodation for several days. The attack rate was 57% (four out of seven family members). Two invasive infections occurred in elderly persons, one of them suffering from underlying cardiac and vascular disease, but the third infection occurred in an otherwise healthy woman without any known risk factor. Although secondary cases are rare in the community, the relative risk of invasive disease among contacts has been reported to vary from 20, when the exposure time was < 24 h/week, to 200 times when it reached ≥ 24 h [17–19].

In recent years, the incidence of invasive *S. pyogenes* disease has increased worldwide. In France, the annual incidence of bacteraemia increased from 1.7 per 100 000 inhabitants in 2002 to 2.7 per 100 000 in 2004, and decreased to 2 per 100 000 in 2007 (http://www.invs.sante.fr). According to the French guidelines, antibioprophylaxis is recommended to persons with increased risk for invasive *S. pyogenes* disease (age > 65, intravenous drug use, cutaneous lesions, chickenpox, underlying pathology, corticotherapy). In addition, when one family member receives antibioprophylaxis, all close contacts should be treated (http://www.sante.gouv.fr/htm/dossiers/cshpf/a_mt_181105_streptococcus.pdf). According to the UK guidelines, if two cases or more occur in the same household within a 30-day period, the entire household should receive chemoprophylaxis [20]. Moreover, household contacts should consult a physician if any pathological sign or symptom appears during the 30 days following the last contact with the index case.

This outbreak, due to a rarely reported *emm*-type 11 strain of *S. pyogenes*, demonstrates the importance of the characterization of the molecular type to prompt application of guidelines for the prevention of secondary cases among household members. Indeed, with the increase of invasive *S. pyogenes* disease in the community, greater numbers of clusters of cases might be expected.

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Transparency Declaration

The authors declare the absence of any relationship or any degree of conflicting or dual interest that may affect professional judgment in relation to the article.

References

1. Daneman N, Green KA, Low DE *et al.* Surveillance for hospital outbreaks of invasive group A streptococcal infections in Ontario, Canada, 1992 to 2000. *Ann Intern Med* 2007; 147: 234–241.
2. Thigpen MC, Richards CL Jr, Lynfield R *et al.* Invasive group A streptococcal infection in older adults in long-term care facilities and the community, United States, 1998–2003. *Emerg Infect Dis* 2007; 13: 852–859.
3. Mihaila-Amrouche L, Bouvet A, Loubinoux J. Clonal spread of emm type 28 isolates of *Streptococcus pyogenes* that are multiresistant to antibiotics. *J Clin Microbiol* 2004; 42: 3844–3846.
4. Raymond J, Schlegel L, Garnier F *et al.* Molecular characterization of *Streptococcus pyogenes* isolates to investigate an outbreak of puerperal sepsis. *Infect Control Hosp Epidemiol* 2005; 26: 455–461.
5. Malhotra-Kumar S, Lammens C, Piessens J, Goossens H. Multiplex PCR for simultaneous detection of macrolide and tetracycline resis-

tance determinants in streptococci. *Antimicrob Agents Chemother* 2005; 49: 4798–4800.
6. O'Loughlin RE, Roberson A, Cieslak PR *et al.* The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. *Clin Infect Dis* 2007; 45: 853–862.
7. Rivera A, Rebollo M, Miro E *et al.* Superantigen gene profile, emm type and antibiotic resistance genes among group A streptococcal isolates from Barcelona, Spain. *J Med Microbiol* 2006; 55: 1115–1123.
8. Ekelund K, Darenberg J, Norrby-Teglund A *et al.* Variations in emm type among group A streptococcal isolates causing invasive or noninvasive infections in a nationwide study. *J Clin Microbiol* 2005; 43: 3101–3109.
9. Creti R, Imperi M, Baldassarri L *et al. emm* types, virulence factors, and antibiotic resistance of invasive *Streptococcus pyogenes* isolates from Italy: what has changed in 11 years? *J Clin Microbiol* 2007; 45: 2249–2256.
10. Malhotra-Kumar S, Lammens C, Chapelle S *et al.* Macrolide- and telithromycin-resistant *Streptococcus pyogenes*, Belgium, 1999–2003. *Emerg Infect Dis* 2005; 11: 939–942.
11. Bingen E, Bidet P, Mihaila-Amrouche L *et al.* Emergence of macrolide-resistant *Streptococcus pyogenes* strains in French children. *Antimicrob Agents Chemother* 2004; 48: 3559–3562.
12. Perez-Trallero E, Montes M, Orden B *et al.* Phenotypic and genotypic characterization of *Streptococcus pyogenes* isolates displaying the MLSB phenotype of macrolide resistance in Spain, 1999 to 2005. *Antimicrob Agents Chemother* 2007; 51: 1228–1233.
13. Nielsen HU, Hammerum AM, Ekelund K *et al.* Tetracycline and macrolide co-resistance in *Streptococcus pyogenes*: co-selection as a reason for increase in macrolide-resistant *S. pyogenes*? *Microb Drug Resist* 2004; 10: 231–238.
14. Luca-Harari B, Darenberg J, Neal S *et al.* Clinical and microbiological characteristics of severe *streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2009; 47: 1155–1165.
15. Gamba MA, Martinelli M, Schaad HJ *et al.* Familial transmission of a serious disease-producing group A streptococcus clone: case reports and review. *Clin Infect Dis* 1997; 24: 1118–1121.
16. Mazon A, Gil-Setas A, Sota de la Gandara LJ, Vindel A, Saez-Nieto JA. Transmission of *Streptococcus pyogenes* causing successive infections in a family. *Clin Microbiol Infect* 2003; 9: 554–559.
17. Davies HD, McGeer A, Schwartz B *et al.* Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A Streptococcal Study Group. *N Engl J Med* 1996; 335: 547–554.
18. Robinson KA, Rothrock G, Phan Q *et al.* Risk for severe group A streptococcal disease among patients' household contacts. *Emerg Infect Dis* 2003; 9: 443–447.
19. Weiss K, Laverdiere M, Lovgren M *et al.* Group A streptococcus carriage among close contacts of patients with invasive infections. *Am J Epidemiol* 1999; 149: 863–868.
20. Health Protection Agency group A Streptococcus Working group. Interim UK guidelines for management of close community contacts of invasive group A streptococcal disease. *Commun Dis Public Health* 2004; 7: 354–361.

Distribution of *emm* types among group A streptococcal isolates from Serbia

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Abstract

This is the first study concerning the molecular epidemiology of group A streptococcus in Serbia and includes 145 isolates from patients with various infections during the period 2001–2007. The *emm* types, superantigen profile and susceptibility pattern were determined. Among 31 *emm* types identified, the most prevalent were *emm*6, *emm*12, *emm*1, and *emm*58. All isolates showed uniform antimicrobial susceptibility to all tested antibiotics, with the exception of tetracycline and erythromycin (41% and 0.7% resistant strains, respectively). Significant heterogeneity of *emm* types was found, with a high frequency of *emm*6 and *emm*58, as well as a considerable prevalence of tetracycline resistance, and a low level of macrolide resistance.

Keywords: *emm* type, resistance, *Streptococcus pyogenes*, superantigens

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Streptococcus pyogenes (group A streptococcus (GAS)) is a common human pathogen that causes a variety of diseases, which differ greatly in severity. An increase in the incidence of severe GAS infection in the late 1980s [1] has prompted surveillance studies of streptococcal diseases around the world. A striking variation in *emm* type distribution in different geographical regions has been observed [2,3]. In addition, different clinical manifestations were related to particular M/*emm* types [4,5]. Since there, there have been no available studies concerning GAS epidemiology in Serbia; the aim here